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CATHODE/CATHOLYTE IMPACTS ON POWER DENSITY AND ANODE PERFORMANCE IN MICROBIAL FUEL CELLS

A Thesis in

Environmental Engineering

by

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ABSTRACT

Microbial fuel cells (MFCs) are biological batteries that have the potential to turn wastewater treatment into an energy-generating process, though more research is needed for them to be feasible on a large scale. Standard cube-reactor shaped MFCs were tested with different cathode chamber conditions (ferricyanide catholytes or air cathodes), and an electrode area-normalized MFC comparison method called the electrode potential slope (EPS) method was used to quantify cell potentials and electrode resistances. Reactors with a ferricyanide catholyte and brush electrodes (FC-B) had the highest maximum power density of 2.46 ± 0.26 Wm⁻². Maximum power densities were lower for MFCs with ferricyanide catholytes using flat carbon paper cathodes with a stirred catholyte (FC-F-S, 1.98 ± 0.28 Wm⁻²), and the same configuration without stirring (FC-F, 1.76 ± 0.12 Wm⁻²). Air cathode MFCs with a 70% porosity diffusion layer (A-70) had higher maximum power densities (1.33 ± 0.14 Wm⁻²) than those with a 30% porosity diffusion layer (A-30, 0.97 ± 0.07 Wm⁻²), but both of these produced lower maximum power densities than the ferricyanide cathode MFCs.

Although the ferricyanide MFCs had higher maximum power densities, using the EPS analysis showed that total electrode resistances were lower for the air cathode MFCs than the ferricyanide MFCs. The sum of the cathode and catholyte resistances in the two types of flat cathode ferricyanide reactors were $36 \pm 0 \text{ m}\Omega \text{ m}^2$ (FC-F-S) and $46 \pm 5 \text{ m}\Omega \text{ m}^2$ (FC-F) compared to $20 \pm 0 \text{ m}\Omega \text{ m}^2$ (A-70) and $28 \pm 6 \text{ m}\Omega \text{ m}^2$ (A-30) for the air cathode MFCs. However, the sum of cathode and catholyte resistances for the brush cathode ferricyanide reactor, FC-B, $(17 \pm 1 \text{ m}\Omega \text{ m}^2)$ was slightly lower than the air cathode reactors, possibly due to improved mass transfer due to increased surface from the 3D brush that was not included in the projected area-based power normalization. These results show that the use of a flat electrode with ferricyanide does not necessarily provide the lowest cathode/catholyte resistances for MFCs.

Unlike the MFC cathodes, the bioanodes of all the reactors performed similarly. The anode resistances appeared to be uninfluenced by the choice of cathode or catholyte in any of these configurations (FC-B, $25 \pm 3 \text{ m}\Omega \text{ m}^2$; FC-F-S, $22 \pm 2 \text{ m}\Omega \text{ m}^2$; FC-F, $22 \pm 2 \text{ m}\Omega \text{ m}^2$; A-70, $17 \pm 1 \text{ m}\Omega \text{ m}^2$; A-30, $21 \pm 1 \text{ m}\Omega \text{ m}^2$), which suggests that anode performance was independent of cathode performance and did not contribute to differences in overall reactor performance.

Using the EPS method, it was shown that the primary benefit of ferricyanide towards higher maximum power is the higher theoretical open circuit potential (361 mV) as compared to the more commonly utilized two-electron transfer oxygen reduction reaction (267-337 mV). If improvements in air cathodes can be made to increase the utilization of four-electron transfer reactions (815 mV), which have a much higher thermodynamic potential than ferricyanide, it follows that reactor maximum power densities will also be higher with the improved air cathodes than with ferricyanide.

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Chapter 1

Introduction

Approximately 30.2 billion kilowatt hours per year, or 0.8% of all the electricity used in the United States, is used for wastewater treatment (Electric Power Research Institute & Water Research Foundation, 2013). Domestic wastewater typically contains more than nine times more chemical potential energy than the equivalent amount of electricity required to treat it (Shizas & Bagley, 2004). Harvesting this energy in the wastewater could transform the treatment system into an energy-positive process. Microbial fuel cells (MFCs) are a type of bioelectrochemical system and are effectively microbial batteries as they degrade organic matter in liquids such as wastewater and produce electricity. In addition, since the wastewater does not have to be aerated they could be used as a method to replace the energy-intensive aeration stage of conventional wastewater treatment (Malaeb et al., 2013). This combination could enable the replacement of an energyconsuming step with an energy-producing step. Bringing MFCs from the lab to implementation in wastewater treatment plants requires metrics to quantify performance. Therefore, comparison of various reactor components and configurations needs to be conducted in a manner that allows direct and quantitative methods in order to improve the effectiveness of MFCs for wastewater treatment and power generation.

1.1 MFC Overview

MFCs contain conductive materials that act as electrodes, which form the anode and cathode of an electrical circuit (Figure 1). The electrodes are connected outside the reactor by a wire with a resistive load, and inside the reactor by a conductive liquid. The liquid in the chamber containing the anode is referred to as the anolyte. The cathode may be located in the same chamber as the anode (and therefore in contact with the anolyte) or in a separate chamber containing a liquid catholyte, which is divided from the anode chamber by a conductive membrane or other separator (Logan et al., 2006).



Figure 1: Operating principles of a two-chamber MFC (Logan et al., 2006)

The anode has exoelectrogenic microbes growing on its surface immersed in the anolyte (e.g. wastewater), which provides the microbes with a source of electrons (acetate or other biodegradable substrates) (Logan et al., 2006). The microbes oxidize the electron donor and transport electrons through respiratory enzymes to generate energy for the cell. Following this, the microbes then transport the electrons externally to a surface outside their outer membrane using different methods, such as conductive pili (Gorby et al., 2006; Reguera et al., 2005). This ability to transfer electrons outside the cell is specific to exoelectrogens and distinct from respiration of electron acceptors such as oxygen and nitrate that occur inside the cell. Some anaerobic microbes have developed the ability to reduce metals (e.g. Fe(III) to Fe(II)), and the exoelectrogens used in

MFCs have expanded this ability and can transfer electrons to a conductive but stable material, such as a carbon electrode. In an MFC, exoelectrogens transfer the electrons directly to the anode itself.

From the anode, the electrons travel through a circuit to reach the cathode. The cathode may be in a liquid catholyte with an electron acceptor such as ferricyanide or may be in contact with air to use oxygen as the electron acceptor. Electrons pass through the cathode and reduce the electron acceptor that is in contact with it. For air cathodes, this is a three-phase reaction: oxygen in the air reacts with the solid conductive catalyst, as well as with liquid water. For liquid catholytes, this reaction is a simpler two-phase reaction, between the solid conductive catalyst and the liquid catholyte containing both water and the final electron acceptor.

1.2 Research Objectives

Early research with MFCs involved an aerated liquid catholyte that was separated from the anolyte by an ion exchange membrane (Bond & Lovley, 2003; Chaudhuri & Lovley, 2003; Oh et al., 2004). This two-chamber aerobic cathode configuration was followed by a single-chamber air cathode configuration, which is composed of a catalyst such as activated carbon, a binder to hold the catalyst to a support such as stainless steel mesh, and a diffusion layer that allows oxygen transfer to the catalyst but prevents liquid leakage (Yang et al., 2015). A cube reactor with an air cathode was first designed in 2004 (Liu & Logan, 2004), and this configuration has since become a standard, allowing for comparisons between reactors due to similar reactor configurations (Yang et al., 2017).

Ferricyanide is often utilized when maintaining strict anaerobic conditions is a priority for testing strictly anaerobic cultures (Logan et al., 2019), in which case other reactor designs are preferable to cube reactors, since cube reactors are not completely sealed from air and thus are not gas tight. Thus, no previous research has been done to directly compare ferricyanide catholytes to these air cathodes in standardized cube reactors. However, ferricyanide is still used as a catholyte for testing with strictly anaerobic cultures in the laboratory (Logan et al., 2019), and it remains important to know how results using ferricyanide can be compared to results using air cathodes. The goal of this study was to compare ferricyanide catholyte to air cathodes in cube MFC, and use the newly developed Electrode Potential Slope (EPS) method to quantify the impacts of the electrodes and solution conductivities on power densities produced using mixed-culture MFCs.

Chapter 2

Literature Review

2.1 MFC Cathodes

The choice of final electron acceptor is a core component of MFC design and the cathode often consists of a conductive cathode exposed either to air or to a liquid solution, the latter of which is referred to as a catholyte. Other than the exposure medium, most MFC cathode material and design considerations are in maximizing conductivity and surface area for a given geometry.

In selecting an electron acceptor, ambient air exposure is a practical and free option for MFCs because oxygen is a good enough electron acceptor and is present at high enough quantities to enable the operation of cathodes of large-scale MFCs (see Table 1). Some exoelectrogenic microbes which have been utilized for MFCs, like many *Geobacter* sp., can tolerate the presence of oxygen, and thus will not be harmed if oxygen crosses over from the cathode region into the anode region where the microbes are growing. However, many exoelectrogens grow better in environments where there is low or no oxygen, which is where they are naturally found, using alternative suitable electron acceptors such as iron (Qu et al., 2012). Some other exoelectrogens are strict anaerobes, which cannot tolerate even the presence of oxygen (Yilmazel et al., 2018). This is a problem because with MFCs using oxygen as an electron acceptor at the cathode, oxygen can cross the membrane from the cathode chamber into the anode chamber, damaging or killing the exoelectrogens. For these anaerobic microbes, a liquid catholyte (often a phosphate buffer solution) containing an alternative electron acceptor such as ferricyanide (hexacyanoferrate Fe(CN)₆³⁻) can be sparged with anaerobic gas to remove oxygen and maintained under strict anaerobic conditions (Logan et al., 2019). Air cathodes are generally considered to be more practical for scaled-up

MFCs, however, since ferricyanide has to be chemically regenerated in order to reuse (Logan et al., 2006).

The oxygen reduction reaction can occur by two different pathways. The first is as a 4electron transfer reaction, which is preferable for power production because of its higher thermodynamic potential. An MFC with an acetate-oxidizing anode $(25^{\circ}C, pH = 7)$ and an oxygenreducing cathode could theoretically achieve a whole cell potential of 815 - (-296) = 1111 mV. However, 2-electron transfer reactions are more commonly achieved with activated carbon air cathodes, although researchers continue to work on improving this performance (Watson et al., 2013; Yang & Logan, 2016). These 2-electron reactions have slightly different potentials, so an MFC with an acetate-oxidizing anode and an oxygen-reducing cathode could theoretically only achieve a whole cell potential of between 267 - (-296) = 563 mV and 337 - (-296) = 663 mV. In comparison, an MFC with the same anode and a ferricyanide reducing cathode could theoretically achieve a whole cell potential of 361 - (-296) = 657 mV. Thus, the performance of air cathodes is dependent on the main pathway used for oxygen reduction relative to the single pathway that occurs with a ferricyanide cathode.

Electrode	Reaction	E _{half-cell} (mV)	Reference
Anode	$2HCO_3^{-} + 9H^+ + 8e^- \rightarrow CH_3COO^- + 4H_2O$	-296	(Rossi et al., 2019)
Cathode	$O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$	815	(Rossi et al., 2019)
	$O_2 + 4e^- + 2H_2O \rightarrow 4OH^-$	815	(Rossi et al., 2019)
	$O_2 + 2e^- + 2H^+ \rightarrow H_2O_2$	281	(Rossi et al., 2019)
	$O_2 + 2e^- + 2H_2O \rightarrow H_2O_2 + 2OH^-$	267	(Rossi et al., 2019)
	$O_2 + 2e^- + H_2O \rightarrow HO_2^- + OH^-$	337	(Rossi et al., 2019)
	$Fe(CN)_6^{3-} + e^- \rightarrow Fe(CN)_6^{4-}$	361	(Logan et al., 2006)

Table 1: Theoretical potentials under typical conditions in MFCs

2.2 Other MFC Components

The other two components of the MFC, the anode and the membrane, also impact MFC performance. For the anode, desirable characteristics include high conductivity and a large specific surface area to maximize the number of microbes that can have direct contact with the surface. Carbon fiber brushes are a common type of anode, but a variety of materials can be used, such as carbon felt, carbon cloth, graphite plates, and titanium (Gu et al., 2017; Logan et al., 2018).

In MFCs having two chambers, a membrane is used to separate the chambers. In this case, a membrane is selected based on its capacity for allowing the desired charge of ion through, while preventing other components from crossing the barrier. For MFCs with ferricyanide in the catholyte, it is desirable to prevent the negatively charged ferricyanide ions from crossing over into the anode chamber, which would be toxic to anode bacteria. Therefore, a cation exchange membrane is used in these reactors. Nafion 117 has been a standard choice for MFCs (Oh et al., 2004), but other membranes such as those manufactured by Selemion (CMV) have been used as less expensive alternatives. The membrane is important because it controls ion transport between the anolyte and catholyte. When the electron donor is oxidized at the anode, the reaction produces electrons, but also releases protons (Table 1). When the electron acceptor is reduced at the cathode, hydroxide ions are produced. The protons can pass through the cation exchange membrane and join with the hydroxide ions to form water, which allows for the overall pH balance of the reaction to remain neutral and completes the electrical circuit. However, localized pH gradients often develop as more abundant cations will usually balance the charge between the two electrolytes (Ye et al., 2016).

2.3 Performance Comparisons of Different MFCs

Since there are many different configurations of MFCs, and many different types of materials can be used as electrodes, electrolytes, and reactors, comparing the performances of material selections in MFCs, as well as the MFCs themselves has historically been difficult (Rossi et al., 2019). The maximum power density, or maximum power normalized by a limiting area, has traditionally been reported and used to compare MFC performance. This area is typically either the smaller electrode's projected or surface area, or the membrane's cross-sectional area (Oh & Logan, 2006). Using the projected area does not account for the increase in reactive surface area available with non-flat electrodes such as brushes, which can contribute to error in MFCs with small electrodes where the projected area is used instead of actual surface area. Additionally, power production can be affected by many other variables which are not accounted for using this method, including relative cathode/anode sizes and electrode separation (Rossi et al., 2019).

Individual abiotic electrodes can also be compared through electrochemical methods. One such method is linear sweep voltammetry (LSV), which can be conducted on individual electrodes or the whole cell. However, LSV requires the use of a potentiostat, which is a specialized, expensive piece of equipment, whereas the EPS method requires nothing more than the use of a multimeter and some resistors.

2.4 Electrode Potential Slope Method

The maximum power density and other information is obtained through a polarization test, where typically the external resistance used in the external circuit is varied and the whole cell potential (difference in potential between the anode and cathode) and individual electrode potentials (difference between the individual electrode and a reference electrode with known standard potential) are recorded once stable. The set resistance and resulting potential data are converted to provide data on power density and potential at different values of the reactor's current density. The use of this polarization data has been standard for MFCs since the early 2000s (Liu et al., 2004; Logan et al., 2006).

A new method was recently developed to quantify electrode performance using electrode polarization data (Rossi et al., 2019). This method, called the electrode potential slope (EPS) method, can be used to calculate area-based resistances and effective half-cell potentials based on the linear region of the polarization data that occurs near peak power. This analysis enables the comparison of individual reactor components, and their individual influences on the maximum power.

With the use of two reference electrodes, three sets of polarization data can be recorded for the whole cell, the anode, and the cathode. These data are commonly visualized as either power density (power density curve) or potential (polarization curve) as functions of current density. When plotted, polarization data each have three characteristic regions for the whole reactor (Figure 2a) and individual electrodes (Figure 2b). The first, at low current density, is a nonlinear region which is dominated by activation losses. The second is a linear region, which is dominated by ohmic losses. The final region is nonlinear, this time defined by mass transfer or biological rate limitations (Rossi et al., 2019).



Figure 2: (a) Representation of polarization data plot for the whole cell, and power density; (b) representations of polarization data plots for individual electrodes

Under ideal conditions, the measured whole cell potential (E_{wc}) is equivalent to the theoretical electromotive force (E_{emf}) , which in turn is equal to the difference between the cathode (E_{cat}) and anode (E_{an}) half-cell reduction potentials (Logan et al., 2006; Rossi et al., 2019), or

$$E_{wc} \equiv E_{emf} = E_{cat} - E_{an} \tag{1}$$

However, there are multiple losses that occur in real systems, preventing the theoretical values from equaling the measured values. These can be included as follows:

$$E_{wc} = E_{emf} - \sum E_{losses} \tag{2}$$

The two types of losses are represented as

$$\sum E_{losses} = \sum \eta + (\sum R_{\Omega})i \tag{3}$$

where η represents electrode overpotentials, including activation losses, concentration losses, microbial metabolic losses, and pH effects. R_{Ω} represents Ohmic losses, which are scaled directly with current, and include solution resistance, membrane resistance (if a membrane is present), and circuit resistance (often, including in the current study, assumed to be negligible). Combining these equations results in

$$E_{wc} = E_{cat} - E_{an} - \left(\sum \eta_{an} + \left|\sum \eta_{cat}\right| + \sum \eta_{other} + \sum R_{\Omega} i\right)$$
(4)

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For the linear portion of polarization data, electrode overpotentials and Ohmic losses are dominant, and other losses such as activation and mass transfer losses (η_{other}) are comparatively negligible (Rossi et al., 2019), producing

$$E_{wc} = E_{cat} - E_{an} - \left(\sum \eta_{an} + \left|\sum \eta_{cat}\right| + \sum R_{\Omega} i\right)$$
(5)

We can represent equation 5 as a linear equation, with overpotentials and losses individually represented as resistances, where R_{an} , R_{cat} , R_{sol} and R_{mem} represent anode, cathode, solution, and membrane resistance, respectively, which produces the equation:

$$E_{wc} = E_{cat,e0} - E_{an,e0} - i(R_{an} + |R_{cat}| + R_{sol} + R_{mem})$$
(6)

In a polarization test, the load on the circuit is changed by changing the external resistance connected between the anode and cathode. E_{cell} can therefore be replaced by this external resistance (R_{ext}) and current to produce

$$iR_{ext} = (E_{cat,e0} - E_{an,e0}) - i(R_{an} + |R_{cat}| + R_{sol} + R_{mem})$$
(7)

Equation 7 can be fit to the linear portion of the plotted polarization data by calculating the slope of the line, as well as estimating the y-intercept of the straight line (Rossi et al., 2019). For the whole cell polarization data, the difference between $E_{cat,e0}$ and $E_{an,e0}$ is the y-intercept of the line, while the sum of the resistances is the slope of the line.

Solution resistance can be calculated for both the analyte and catholyte using the solution conductivity (σ) and the distance between the electrodes (*L*) as

$$R_{sol} = \frac{L}{\sigma} \tag{8}$$

To obtain the individual electrode polarization data, lines can be fit to the individual electrode polarization curves:

$$iR_{ext} = E_{an,e0} - i(R_{an}) \tag{9}$$

$$iR_{ext} = \mathcal{E}_{cat,e0} - i(R_{cat}) \tag{10}$$

Therefore, the y-intercept of each line would be the expected open circuit anode or cathode potential $(E_{an,e0} \text{ or } E_{cat,e0})$, and the slope of each line represents the resistance of that electrode $(R_{an} \text{ or } R_{cat})$ (Figure 3).

The membrane resistance could be obtained using electrochemical impedance spectroscopy (EIS) on an abiotic reactor, in the presence and absence of a membrane which would allow calculation of the solution and membrane resistances (Min et al., 2005; Rossi et al., 2019). If the resistance is measured with only the membrane and solution, the solution resistances can be calculated using equation 8, and thus the membrane resistance could be obtained by difference. Alternatively, the membrane resistance can be assumed to be the difference between the sum of the individual resistances and the measured internal resistance of the reactor (R_{int}) based on the whole cell polarization data.

$$R_{mem} = R_{int} - (R_{an} + R_{cat} + R_{sol}) \tag{11}$$

The difference between the experimentally measured open circuit potential (E_{m0}) and the calculated expected open circuit potential (E_{e0}) is due to the losses left out of this model that do not change linearly with respect to current, such as activation and concentration losses (Figure 3).



Figure 3: Components of polarization curves for (a) whole cell and (b) individual electrodes, with linear regions bolded, and slopes and theoretical intercepts marked

Two previous studies have provided quantitative results on the values for the resistances and working electrode potentials of bioelectrochemical systems. The first was the paper detailing the EPS method, which compared two types of air cathodes in MFCs fed with two types of media (Rossi et al., 2019), the results of which are reported in Table 2. In the second study the EPS method was used to analyze microbial electrolysis cells (MECs), a different type of bioelectrochemical system (Cario et al., 2019). In an MEC, the potential generated by the microbial anode is supplemented by an external energy source, providing enough energy to split water at the cathode and produce hydrogen gas. In the results reported by Rossi et al. (2019), the difference in conductivity between wastewater (lower conductivity) and buffered solution with acetate (higher conductivity) resulted in very different solution resistances. However, anode and cathode resistances also appeared to be grouped by media type, rather than electrode type, and overall reactor performance (current densities reached, maximum power density achieved) followed this grouping as well. This suggested that solution conductivity was the most important factor in the reactor internal resistance, and potentially influenced the anode and cathode resistances as well. If the solution resistance did indeed impact the measurements of anode and cathode resistance, this method may need more development to help separate the anode and cathode resistances from the solution resistance.

Cathode (diffusion layer % porosity)	70%	30%	70%	30%	
Electron Donor	Acetate	Acetate	Wastewater	Wastewater	
$R_{int, from whole cell}$ (m Ω m ²)	41 ± 1	39 ± 4	223 ± 16	249 ± 12	
<i>R_{int, from electrodes}</i> (mΩ m ²)	39.4	37	216	243	
<i>R_{sol}</i> (mΩ m²)	14	14	87	87	
<i>R_{an}</i> (mΩ m²)	10.6 ± 0.5	11 ± 1	75 ± 9	78 ± 2	
<i>R_{cat}</i> (mΩ m²)	14.8 ± 0.9	12 ± 5	54 ± 7	78 ± 14	
<i>E_{wc, m0}</i> (mV)	664 ± 11	669 ± 4	617 ± 2	632 ± 2	
<i>E_{wc, e0}</i> (mv)	531 ± 5	467 ± 22	574 ± 22	574 ± 16	
<i>E_{cat, m0}</i> (mV)	399 ± 11	401 ± 2	337 ± 1	350 ± 2	
<i>E_{cat, e0}</i> (mV)	271 ± 6	205 ± 27	308 ± 10	313 ± 18	
<i>E_{an, m0}</i> (mv)	-	-	-280 ± 3	-280 ± 3	
<i>E_{an, e0}</i> (mV)	-260 ± 3	-262 ± 7	-266 ± 13	-262 ± 3	
Current Density Range (A/m ²)	3.0 – 9.0	3.0 - 9.0	0.5-2.0	0.5-2.0	
Maximum Power Density Measured (W/m ²)	1.71 ± 0.08	1.38 ± 0.04	0.34 ± 0.01	0.31 ± 0.01	
Maximum Power Density Calculated (W/m ²)	1.71	1.4	0.37	0.33	

Table 2: EPS results for air cathode cube reactors (Rossi et al., 2019)

Chapter 3

Methods

3.1 Standard Reactor Conditions

Four cubic MFCs were set up following previous standardized methods (Logan et al., 2007) using polycarbonate reactors with cylindrical inside chambers 3 cm diameter and 4 cm length (liquid volume of 26 mL). Reactor chambers were bolted together with end plates holding the electrodes. Anodes were graphite carbon brushes (2.5 cm diameter, with a brush length of 2.5 cm), wrapped and held with titanium wire (Mill-Rose, Mentor, OH). Brushes were pretreated at 450°C for 30 minutes to remove any impurities and increase surface area prior to use.

The anolyte was 50 mM phosphate buffer (4.58 g/L Na₂HPO₄, 2.45 g/L NaH₂PO₄, 0.31 g/L NH₄Cl, and 0.13 g/L KCl) with 1 g/L sodium acetate as electron donor, and 12.5 mL/L of concentrated trace minerals and 5 mL/L of concentrated vitamins (Lovley & Phillips, 1988; Yang et al., 2017). The average conductivity of the anolyte was measured as 7.5 mS/cm. Reactors were inoculated with effluent from other MFCs that were being fed the same medium (having originally been inoculated with wastewater). During inoculation of the reactors in this study, effluent from the other reactors was mixed with an equal amount of fresh anolyte medium. After the inoculation period, the anolyte was always completely replaced with fresh anolyte. Catholyte, when used, was 50 mM phosphate buffer with 50 mM potassium ferricyanide added as electron acceptor (conductivity 23.5 mS/cm). Anolyte and catholyte were prepared frequently and stored at 4°C to prevent microbial growth during storage.

Anolyte and catholyte were replaced at the same time, whenever the measured potential dropped below 10% of the cycle maximum potential, as measured by a multimeter with a data acquisition system (Keithley Instruments Model 2700, Cleveland, OH, USA). Reactors were fully

acclimated before testing was begun, so that the current generation pattern throughout the feeding cycle was repeated and stable for multiple cycles. Reactors were operated with an external resistance of 1000Ω in a constant temperature room set at 30° C.

3.2 Cathode Configurations

The MFCs were set up with identical anode chamber components and conditions, but different cathode chamber components and conditions. Two groups of cathodes were used: air cathodes utilizing oxygen gas as the final electron acceptor, and ferricyanide cathodes utilizing ferricyanide dissolved in a liquid solution (catholyte) as the final electron acceptor. The air cathodes were manufactured by VITO (Mol, Belgium), containing stainless steel mesh, activated carbon, and a polytetrafluoroethylene (PTFE) diffusion layer with either 30% or 70% porosity (referred to as A-30 and A-70, respectively). The cathodes used with ferricyanide (FC) were either flat carbon paper (F) that were used with stirring (FC-FS) or no stirring (FC-F), or graphite carbon brushes (FC-B) without stirring. The catholyte for FC-FS was stirred at a constant rate via a magnetic stir bar, moved by an external stir plate. The liquid catholyte was composed of 50 mM phosphate buffer (to maintain consistency with the anolyte) with 50 mM potassium ferricyanide added as electron acceptor.

All tests were done in duplicate: a set of cubic MFCs was used for all of the ferricyanide catholyte tests, and another set of cubic MFCs was tested with the air cathodes. Reactors with air cathodes had one chamber (anode chamber), but reactors with ferricyanide cathodes used two chambers separated by a cation exchange membrane (Selemion, Bellex International Corporation, Wilmington, DE). Before each polarization test, a Ag/AgCl reference electrode (model RE-5B, BASi; 0.209 V vs a standard hydrogen electrode) was positioned in each reactor chamber (Figure

4, long grey rectangles indicate reference electrode positions). Distance measurements between anodes, cathodes, and reference electrodes are reported in Table A1.





Ferricyanide Catholyte, Stirred Flat Cathode (Carbon Paper) (FC-F-S)



Air Cathode (70% or 30% porosity) No Catholyte (A-70, A-30)

Figure 4: MFC reactor side views, with ferricyanide catholyte (FC) or air cathodes (A). Long grey rectangles indicate reference electrode positions.

3.3 Polarization Tests

For polarization tests, reactors were acclimated to lower set resistances (20-500 Ω), with correspondingly higher current densities, for two days prior to polarization tests. The whole cell potential was measured between the anode and cathode. The potential of each individual electrode was measured with respect to the reference electrode in the same reactor chamber. Before the start of the polarization test, the reactor analyte and catholyte were replaced with fresh liquids. For the open circuit potential measurement, reactors were disconnected from the circuit (such that the anode and cathode were not connected by wires). After two hours, the whole cell and individual electrode potentials were measured. Following these measurements, the resistance was incrementally lowered with 20 minutes at each resistance to ensure a stable value before potential measurements were taken. Resistances used were: 1000Ω , 500Ω , 200Ω , 100Ω , 75Ω , 50Ω , and 20Ω . Any polarization test showing power overshoot was discarded; reactors were operated at 1000Ω for another week, and longer acclimation periods at lower resistances were used to prepare the reactors for additional polarization tests. Power overshoot is where the power density curve doubles back to lower current densities as the external resistance was decreased, instead of continuing to increase (Hong et al., 2011).

3.4 Data Analysis

Current density (A/m²) was calculated as i=V/RA, and power density (mW/m²) as P = V²/RA, where V is the potential difference measured (either by the Keithley multimeter with data acquisition system or manually with a multimeter), R the external resistance, and A the projected (cross-sectional) area of the cathode (7 cm² for all cathodes). Electrode potentials measured vs. reference electrodes were corrected to remove the influence of the solution resistance in the distance between the two electrodes (Logan et al., 2018).

Polarization data were analyzed using the EPS method as described in Chapter 2 (Rossi et al., 2019). The points chosen for the linear regressions were defined as the points which included and surrounded the point at maximum power density, which also appeared to be visibly in the linear portion of the data.

The solution conductivity and the distances between electrodes were used to calculate solution resistance for both anolyte and catholyte (Table A1). The remaining unmeasured or uncalculated resistance was that of the membrane, which was calculated by taking the difference between the sum of the other resistances and the whole cell resistance.

Chapter 4

Results and Discussion

4.1 Reactor Comparisons using Polarization and Power Density Curves

Polarization test results showed that ferricyanide cathode reactors had higher power densities than the air cathode reactors, but each cathode type resulted in a different maximum power density (Figure 5a). Reactors with brush cathodes and ferricyanide catholyte (FC-B) had the highest maximum power density of 2.46 ± 0.26 Wm⁻². This was followed by reactors with flat cathodes and stirred catholyte (FC-F-S, 1.98 ± 0.28 Wm⁻²), and then by reactors with flat cathodes and unstirred catholyte (FC-F, 1.76 ± 0.12 Wm⁻²). For the air cathode reactors, the 70% porosity cathode (A-70) had higher maximum power densities (1.33 ± 0.14 Wm⁻²) than the less porous 30% porosity cathode (A-30, 0.97 ± 0.07 Wm⁻²), but these were both lower than the maximum power densities achieved by the ferricyanide cathode reactors.

The ferricyanide cathode reactors generally had higher whole cell potentials (max, Figure 5b: FC-B, $778 \pm 31 \text{ mV}$; FC-F-S, $812 \pm 1 \text{ mV}$; FC-F, $798 \pm 12 \text{ mV}$) than the air cathode reactors (Figure 5b: A-70, $620 \pm 24 \text{ mV}$; A-30, $685 \pm 22 \text{ mV}$) as well (Figure 5b). However, there was a steep drop in potential during the polarization test for the flat cathode with unstirred catholyte (FC-F), which resulted in this cathode configuration obtaining the lowest maximum current density of all the reactors, including the air cathode reactors.

The anode potentials as a function of current density were all approximately the same for all the reactor configurations (Figure 5c). These results indicate that despite different cathodes and current densities, the resulting anode conditions were the same. Air cathodes can allow oxygen to enter the anode chamber, whereas the ferricyanide liquid maintains anaerobic conditions. However, the anodes in this study did not show a corresponding difference in performance. It may be that with the mixed culture of the anodes, that other microbes consumed oxygen before it negatively impacted the anode microorganisms. Alternatively, the cube reactors were not sealed tightly, and the aerobic anolyte initially contained some dissolved oxygen, so it could be that the baseline concentration of oxygen in solution was high enough that there wasn't a noticeable impact based on the cathode type.

Activation losses were higher for air cathodes than for ferricyanide cathodes, which is shown by the steeper initial curved region of the air cathode polarization curves as compared to the more gradual ferricyanide cathode curves (Figure 5c). Additionally, there were concentration losses associated with the flat cathode with unstirred ferricyanide, shown by a sharp drop at higher current densities of the cathode polarization curve (Figure 5c). Stirring the ferricyanide catholyte or changing out the electrode for an electrode with larger surface area decreased these losses.



Figure 5: (a) Power density, polarization curves – (b) whole-cell and (c) individual electrodes

Reactors with ferricyanide cathodes had higher cathode potentials than those with air cathodes (Figure 5c). The reactors with flat cathodes and unstirred catholyte (FC-F) had the lowest maximum power density of all the ferricyanide reactors, and they showed a steep drop in potential with higher current densities in the whole cell and cathode polarization curves. This portion of the polarization curve at high current densities can be associated with mass transfer limitations (Rossi et al., 2019), and since the drop was visible in the cathode curve but not the anode curve, mass

transfer of ferricyanide in the catholyte was suspected to be causing the limitation. The catholyte chambers of these reactors were stirred to reduce this limitation (FC-FS). After stirring the ferricyanide, the drop in cathode potential was less steep, which corresponded with a less steep drop in the whole cell potential, and an increase in maximum power density. With the brush cathodes, the reactors reached the highest maximum power density of all configurations tested. The brush cathode ferricyanide reactor may have had the highest power density because of the large increase in surface area of the brush cathode compared to the flat cathode, since the current density and power density calculations are based on projected area, not actual electrode surface area.

4.2 Reactor Comparisons Using EPS Method

4.2.1 Open Circuit Potentials from Linear Regressions

The experimental open circuit cathode potentials for the ferricyanide cathode reactors, calculated from linear regressions using the y-intercept values (Figure 6), were all very similar, ranging from 755 to 773 mV (Figure 7, Table A2). The air cathode experimental open circuit potentials were much lower than the ferricyanide cathode reactors but were similar to one another (A-70, 517 ± 6 mV; 502 ± 25 mV). This suggests a fundamental difference between the ferricyanide catholyte reactors and the air cathode reactors, without much of a difference from variations within these categories.



Figure 6: Linear regressions on whole cell polarization data



Figure 7: Y-intercept values for whole cell linear regressions, expressed as potentials

The open circuit potentials for the cathodes, calculated from the linear regressions (Figure 8), were split into two groups by cathode category, where the ferricyanide cathode reactors (Figure 9, Table A2: FC-B, $511 \pm 5 \text{ mV}$; FC-F-S, $488 \pm 2 \text{ mV}$; FC-F, $502 \pm 22 \text{ mV}$) had a much higher intercept than the air cathode reactors (A-70, $237 \pm 1 \text{ mV}$; A-30, $230 \pm 23 \text{ mV}$). Within each of these two groups, the y-intercept values were fairly similar to one another. The anodes were all very similar (Figure 8, Figure 9, Table A2: FC-B, $-273 \pm 25 \text{ mV}$; FC-F-S, $-293 \pm 14 \text{ mV}$; FC-F, $-289 \pm 11 \text{ mV}$; A-70, $-278 \pm 3 \text{ mV}$; A-30, $-272 \pm 2 \text{ mV}$). The open circuit potential represents the chemical potential of the electron acceptor. Oxygen is the final electron acceptor for all the air cathodes, and ferricyanide is 361 mV, which is higher than that of the two-electron transfer reactions for oxygen (267-337 mV), which accounts for the higher open circuit potential for these cathodes (Table 2). As the whole cell open circuit potential depends on both the anode and cathode, these values are also separated by cathode category.



Figure 8: Linear regressions on anode and cathode polarization data



Figure 9: Y-intercept values for electrode linear regressions, expressed as potentials

4.2.2 Resistances from Linear Regressions

The whole cell polarization slope coefficient, or overall resistance, was lower for the flat cathode reactor configuration with a stirred catholyte (Figure 10, Table A3: FC-F-S, $73 \pm 3 \text{ m}\Omega$ m²) compared to the reactor configuration with catholyte that was not stirred (FC-F, $84 \pm 8 \text{ m}\Omega$ m²). The cathode resistance was even lower when the brush electrode was substituted for the flat electrode (FC-B, $62 \pm 4 \text{ m}\Omega \text{ m}^2$). For the air cathode reactors, the overall resistance was lower for the reactor with higher porosity (Figure 10, Table A3: A-70, $51 \pm 1 \text{ m}\Omega \text{ m}^2$; A-30, $62 \pm 6 \text{ m}\Omega \text{ m}^2$).



Figure 10: Whole cell coefficient values for linear regressions, expressed as resistances

The absolute values of the slopes from the linear regressions of the electrode polarization data were used to calculate the resistances for each electrode (Figure 11). The trend in cathode resistances varied in a similar fashion to that observed with whole cell resistances. The resistance was lower for the flat cathode reactor configuration with stirred catholyte (Figure 11, Table A3: FC-F-S, $19 \pm 0 \text{ m}\Omega \text{ m}^2$) compared to the reactor configuration with unstirred catholyte (FC-F, 22 $\pm 2 \text{ m}\Omega \text{ m}^2$), and the brush electrode resistance was lower than both of these (FC-B, $11 \pm 1 \text{ m}\Omega \text{ m}^2$). The air cathode resistances were in the same overall range as the ferricyanide cathode resistances, but they were different from one another (Figure 11, Table A3: A-70, $20 \pm 0 \text{ m}\Omega \text{ m}^2$; A-30, $28 \pm 6 \text{ m}\Omega \text{ m}^2$). The brush cathode had the lowest resistance of all the configurations tested.

The anode resistances were not significantly different from one another, either by cathode type (air vs ferricyanide), or specific cathode group (Figure 11, Tables A5-A8: FC-B, $25 \pm 3 \text{ m}\Omega$ m²; FC-F-S, $22 \pm 2 \text{ m}\Omega$ m²; FC-F, $22 \pm 2 \text{ m}\Omega$ m²; A-70, $17 \pm 1 \text{ m}\Omega$ m²; A-30, $21 \pm 1 \text{ m}\Omega$ m²), which continues to suggest that anode performance was independent of cathode and did not contribute to overall reactor performance differences.



Figure 11: Electrode coefficient values for linear regressions, expressed as resistances

4.2.3 Summary of All Resistances

Anolyte, catholyte, and membrane resistances were calculated as described in the methods section, and are shown along with the electrode resistances, compared to the maximum power density (Figure 12a) and overall whole cell resistance (Figure 12b). The membrane resistance was calculated as the difference between the measured whole cell resistance, and the sum of the other measured/calculated resistances. However, there were cases where the sum of the resistances except the membrane resistance was larger than the measured whole cell resistance. For these cases, the difference is shown on the plot as "negative membrane" resistance (Figure 12b). Additionally, the difference in these values was greater for the air cathode reactors (which lack membranes) than for the ferricyanide cathode reactors (which have membranes). The magnitudes of these differences are very small, and therefore are likely due to experimental error, rather than being due to actual membrane resistances. The membrane used in the ferricyanide reactors is known to have low resistance (AGC Engineering Co., 2020), so it is likely that experimental error was larger than the resistances, leading to the membrane resistance not being measurable in these tests.

The reactors with the highest resistances were not necessarily the same as the ones with the lowest maximum power densities (Figure 12a). The flat cathode with unstirred catholyte had the highest total resistance (FC-F, $84 \pm 8 \text{ m}\Omega \text{ m}^2$) but was in the middle of the range of maximum power densities ($1.8 \pm 0.1 \text{ W m}^{-2}$) among MFCs with different cathode conditions (range 1.0-2.5 W m⁻²). The air cathode with 70% porosity had the lowest total resistance (A-70; $51 \pm 1 \text{ m}\Omega \text{ m}^2$) but had also had a maximum power density ($1.3 \pm 0.1 \text{ W m}^{-2}$) in the middle of the range (range 1.0-2.5 W m⁻²). The reactor configuration with the highest maximum power density was the brush cathode with ferricyanide catholyte (FC-B, $2.5 \pm 0.3 \text{ W m}^{-2}$), which was towards the middle of the range of total resistances ($62 \pm 4 \text{ m}\Omega \text{ m}^2$, range 51-84 m $\Omega \text{ m}^2$). These results suggest that overall reactor resistance is not the only factor that impacts maximum power densities.



Figure 12: (a) Area-normalized resistances in all reactors along with maximum power densities, (b) Area-normalized resistances in all reactors compared to measured whole cell resistances

Comparing the resistance categories of solution, electrode, and membrane, the electrode resistances were larger than the other two resistances. Solution resistances were also significant contributors to overall internal resistance, which suggests that decreasing solution resistance could be another potential method of reducing reactor internal resistance and improving maximum power densities. These results agree with Rossi et al. (2019), whose results indicate that reactors with low-conductivity electrolytes will perform much more poorly than reactors with high-conductivity electrolytes, and that solution resistance is often the largest factor to reactor internal resistance, especially for reactors with low-conductivity solutions.

Chapter 5

Conclusions

This was the first time that the EPS method had been used to analyze MFCs containing ferricyanide catholyte. The sum of the cathode and catholyte resistances in the flat cathode ferricyanide reactors (FC-F-S, $36 \pm 0 \text{ m}\Omega \text{ m}^2$; FC-F, $46 \pm 5 \text{ m}\Omega \text{ m}^2$) were generally greater than the cathode resistances for the air cathode reactors that did not have a catholyte (A-70, $20 \pm 0 \text{ m}\Omega \text{ m}^2$; A-30, $28 \pm 6 \text{ m}\Omega \text{ m}^2$). The sum of cathode and catholyte resistances for the brush cathode ferricyanide reactor, FC-B, $(17 \pm 1 \text{ m}\Omega \text{ m}^2)$ was slightly lower than the air cathode reactors. This comparison shows that the use of a solid electrode with ferricyanide does not necessarily provide the lowest cathode/catholyte resistances for microbial fuel cells. Therefore, the primary benefit of ferricyanide for generating a higher maximum power density compared to the air cathode MFCs is the higher open circuit potential of ferricyanide (361 mV) as compared to the two-electron transfer oxygen reduction reaction (267-337 mV). If improvements in air cathodes can be made to increase the utilization of the four-electron transfer reactions (815 mV), which has a higher thermodynamic potential than ferricyanide, it might be possible to increase power densities of air cathodes relative to those with ferricyanide catholytes.

The anode chambers all had similar configurations, and anode and anolyte resistances were all very similar, despite the reactors having different cathodes, maximum power densities, and other resistances. These results suggest that the anode and anolyte resistances were not affected by the performance of the cathode or catholyte with which they were paired. If the anode was affected by the cathode or catholyte, testing of anode improvements would be cathode-dependent, and would therefore have to be re-done with each new cathode improvement to better understand the capabilities of the anode microbiome. Cathode-dependent anodes could be problematic in the potential situation that researchers are eventually able to utilize the four-electron transfer reaction, and power densities with air cathodes are larger than those with ferricyanide cathodes. The effects of cathode type and anode microbiome community would be intertwined, and it could be difficult to compare performance of, for example, strict anaerobic exoelectrogens requiring worseperforming non-air cathodes, to that of an oxygen-tolerant anode microbiome that could be paired with the better-performing air cathode. However, the similarity of anode performance and resistance despite differing cathode conditions in these results suggests that analyses of anodes may be able to be conducted and compared without having to consider negative performance impacts from the cathode and catholyte they are paired with.

Literature Cited

- AGC Engineering Co. (2020). Selemion ion-exchange membranes. https://www.agec.co.jp/eng/product/selemion/
- Bond, D. R., & Lovley, D. R. (2003). Electricity production by *Geobacter sulfurreducens* attached to electrodes. *Applied and Environmental Microbiology*, 69(3), 1548– 1555. https://doi.org/10/fkh
- Cario, B. P., Rossi, R., Kim, K.-Y., & Logan, B. E. (2019). Applying the electrode potential slope method as a tool to quantitatively evaluate the performance of individual microbial electrolysis cell components. *Bioresource Technology*, 287, 121418. https://doi.org/10/gf2pzn
- Chaudhuri, S. K., & Lovley, D. R. (2003). Electricity generation by direct oxidation of glucose in mediatorless microbial fuel cells. *Nature Biotechnology*, 21(10), 1229– 1232. https://doi.org/10/ddnhft
- Electric Power Research Institute, & Water Research Foundation. (2013). *Electricity use and management in the municipal water supply and wastewater industries* (No. 3002001433; p. 194).
- Gorby, Y. A., Yanina, S., McLean, J. S., Rosso, K. M., Moyles, D., Dohnalkova, A.,
 Beveridge, T. J., Chang, I. S., Kim, B. H., Kim, K. S., Culley, D. E., Reed, S. B.,
 Romine, M. F., Saffarini, D. A., Hill, E. A., Shi, L., Elias, D. A., Kennedy, D. W.,
 Pinchuk, G., ... Fredrickson, J. K. (2006). Electrically conductive bacterial
 nanowires produced by *Shewanella oneidensis* strain MR-1 and other

microorganisms. *Proceedings of the National Academy of Sciences*, 103(30), 11358–11363. https://doi.org/10/fgb4bf

- Gu, Y., Ying, K., Shen, D., Huang, L., Ying, X., Huang, H., Cheng, K., Chen, J., Zhou,
 Y., Chen, T., & Feng, H. (2017). Using sewage sludge pyrolytic gas to modify
 titanium alloy to obtain high-performance anodes in bio-electrochemical systems. *Journal of Power Sources*, 372, 38–45. https://doi.org/10/gcsdd5
- Hong, Y., Call, D. F., Werner, C. M., & Logan, B. E. (2011). Adaptation to high current using low external resistances eliminates power overshoot in microbial fuel cells. *Biosensors and Bioelectronics*, 28(1), 71–76. https://doi.org/10/dtfwxs
- Liu, H., & Logan, B. E. (2004). Electricity generation using an air-cathode single chamber microbial fuel cell in the presence and absence of a proton exchange membrane. *Environmental Science & Technology*, 38(14), 4040–4046. https://doi.org/10.1021/es0499344
- Liu, H., Ramnarayanan, R., & Logan, B. E. (2004). Production of electricity during wastewater treatment using a single chamber microbial fuel cell. *Environmental Science & Technology*, 38(7), 2281–2285.
- Logan, B. E., Cheng, S., Watson, V., & Estadt, G. (2007). Graphite fiber brush anodes for increased power production in air-cathode microbial fuel cells. *Environmental Science & Technology*, 41(9), 3341–3346. https://doi.org/10/d3jckx
- Logan, B. E., Hamelers, B., Rozendal, R. A., Schröder, U., Keller, J., Freguia, S.,
 Aelterman, P., Verstraete, W., & Rabaey, K. (2006). Microbial fuel cells:
 Methodology and technology. *Environmental Science & Technology*, 40(17),
 5181–5192. https://doi.org/10.1021/es0605016

- Logan, B. E., Rossi, R., Ragab, A., & Saikaly, P. E. (2019). Electroactive microorganisms in bioelectrochemical systems. *Nature Reviews Microbiology*, 17, 307–319. https://doi.org/10/gfwrrg
- Logan, B. E., Zikmund, E., Yang, W., Rossi, R., Kim, K.-Y., Saikaly, P. E., & Zhang, F. (2018). Impact of ohmic resistance on measured electrode potentials and maximum power production in microbial fuel cells. *Environmental Science & Technology*, *52*(15), 8977–8985. https://doi.org/10/gd4nc5
- Lovley, D. R., & Phillips, E. J. P. (1988). Novel mode of microbial energy metabolism:
 Organic carbon oxidation coupled to dissimilatory reduction of iron or
 manganese. *Applied and Environmental Microbiology*, 54(6), 1472–1480.
- Malaeb, L., Katuri, K. P., Logan, B. E., Maab, H., Nunes, S. P., & Saikaly, P. E. (2013).
 A hybrid microbial fuel cell membrane bioreactor with a conductive ultrafiltration membrane biocathode for wastewater treatment. *Environmental Science & Technology*, 47(20), 11821–11828. https://doi.org/10.1021/es4030113
- Min, B., Cheng, S., & Logan, B. E. (2005). Electricity generation using membrane and salt bridge microbial fuel cells. *Water Research*, 39(9), 1675–1686. https://doi.org/10/d399nr

Oh, S.-E., & Logan, B. E. (2006). Proton exchange membrane and electrode surface areas as factors that affect power generation in microbial fuel cells. *Applied Microbiology and Biotechnology*, 70(2), 162–169. https://doi.org/10.1007/s00253-005-0066-y

- Oh, S.-E., Min, B., & Logan, B. E. (2004). Cathode performance as a factor in electricity generation in microbial fuel cells. *Environmental Science & Technology*, 38(18), 4900–4904. https://doi.org/10/b3gbw4
- Qu, Y., Feng, Y., Wang, X., & Logan, B. E. (2012). Use of a coculture to enable current production by *Geobacter sulfurreducens*. *Applied and Environmental Microbiology*, 78(9), 3484–3487. https://doi.org/10/gfc4q9
- Reguera, G., McCarthy, K. D., Mehta, T., Nicoll, J. S., & al, et. (2005). Extracellular electron transfer via microbial nanowires. *Nature; London*, 435(7045), 1098– 1101. https://doi.org/10/c65x78
- Rossi, R., Cario, B. P., Santoro, C., Yang, W., Saikaly, P. E., & Logan, B. E. (2019).
 Evaluation of electrode and solution area-based resistances enables quantitative comparisons of factors impacting microbial fuel cell performance. *Environmental Science & Technology*, *53*(7), 3977–3986. https://doi.org/10/gfwrq9
- Shizas, I., & Bagley, D. M. (2004). Experimental determination of energy content of unknown organics in municipal wastewater streams. *Journal of Energy Engineering*, 130(2), 45–53. https://doi.org/10/dwrfmv
- Watson, V. J., Nieto Delgado, C., & Logan, B. E. (2013). Influence of chemical and physical properties of activated carbon powders on oxygen reduction and microbial fuel cell performance. *Environmental Science & Technology*, 47(12), 6704–6710. https://doi.org/10/gfc8zv
- Yang, W., Kim, K.-Y., & Logan, B. E. (2015). Development of carbon free diffusion layer for activated carbon air cathode of microbial fuel cells. *Bioresource Technology*, 197, 318–322. https://doi.org/10/gfc82v

- Yang, W., Kim, K.-Y., Saikaly, P. E., & Logan, B. E. (2017). The impact of new cathode materials relative to baseline performance of microbial fuel cells all with the same architecture and solution chemistry †. *Energy & Environmental Science*, 10, 1025–1033. https://doi.org/10.1039/C7EE00910K
- Yang, W., & Logan, B. E. (2016). Immobilization of a metal-nitrogen-carbon catalyst on activated carbon with enhanced cathode performance in microbial fuel cells. *ChemSusChem*, 9(16), 2226–2232. https://doi.org/10/f3qpnh
- Ye, Y., Zhu, X., & Logan, B. E. (2016). Effect of buffer charge on performance of aircathodes used in microbial fuel cells. *Electrochimica Acta*, 194, 441–447. https://doi.org/10.1016/j.electacta.2016.02.095
- Yilmazel, Y. D., Zhu, X., Kim, K.-Y., Holmes, D. E., & Logan, B. E. (2018). Electrical current generation in microbial electrolysis cells by hyperthermophilic archaea *Ferroglobus placidus* and *Geoglobus ahangari*. *Bioelectrochemistry*, 119, 142– 149. https://doi.org/10/gcscx5

Appendix

Reference Data Tables

	Anode-Reference	Anode-Membrane	Cathode-Reference	Cathode-
				Membrane
	[m]	[m]	[m]	[m]
FC-B	0	0.010, 0.014	0	0.040
FC-F-S	0	0.010, 0.014	0.030	0.013, 0.014
FC-F	0	0.010, 0.014	0.030	0.013, 0.014
A-70	0	0.012, 0.013	0.010	0
A-30	0	0.012, 0.013	0.010	0

Table A1: Electrode Spacings (for cells with two values, one reactor for each distance)

Table A2: Y-intercept values for linear regressions (units of mV)

	Anode	Cathode	Whole Cell
	[mV]	[mV]	[mV]
FC-B	-273 ± 18	511 ± 5	773 ± 25
FC-F-S	-293 ± 14	488 ± 2	755 ± 18
FC-F	-289 ± 11	502 ± 22	763 ± 36
A-70	-278 ± 3	237 ± 1	517 ± 6
A-30	-272 ± 2	230 ± 23	502 ± 25

Table A3: Coefficient absolute values for linear regressions, expressed as resistances

	Anode Resistance	Cathode Resistance	Whole Cell Resistance
	[mΩ m²]	$[m\Omega m^2]$	$[m\Omega m^2]$
FC-B	25 ± 3	11 ± 1	62 ± 4
FC-F-S	22 ± 2	19 ± 0	73 ± 3
FC-F	22 ± 2	29 ± 5	84 ± 8
A-70	17 ± 1	20 ± 0	51 ± 1
A-30	21 ± 1	28 ± 6	62 ± 6

	Anolyte	Catholyte	Membrane
	$[m\Omega m^2]$	$[m\Omega m^2]$	$[m\Omega m^2]$
FC-B	19 ± 0	6 ± 0	2 ± 0
FC-F-S	16 ± 1	17 ± 0	-1 ± 0
FC-F	15 ± 0	17 ± 0	1 ± 0
A-70	17 ± 0	0 ± 0	-3 ± 0
A-30	17 ± 0	0 ± 0	-3 ± 0

Table A4: Calculated reactor component resistances

Table A5: ANOVA for anode slopes, reactors grouped by cathode category (A or F)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
current density	1	23732.1	23732.1	95.1143	6.94E-08
reactor	1	336.8	336.8	1.3497	0.26348
current density: reactor	1	831.2	831.2	3.3314	0.08795
Residuals	15	3742.7	249.5		

Table A6: Pairwise comparison of anode slopes, reactors grouped by cathode category (A or F)

Cathode Categories	estimate	SE	df	t.ratio	p.value
A - F	-10.9	6	15	-1.825	0.0879

Table A7: ANOVA for anode slopes, reactors grouped by cathode group

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
current density	1	23732.1	23732.1	901.834	2.46E-10
cathode group	4	4444.7	1111.2	4.22E+01	7.77E-06
current density : cathode group	4	229.2	57.3	2.177	0.1528
Residuals	9	236.8	26.3		

Cathode Groups	estimate	SE	df	t.ratio	p.value
FC-B - FC-F-S	2.545	2.68	9	0.95	0.8705
FC-B - FC-F	2.426	3.69	9	0.658	0.9607
FC-B - A-70	8.01	2.74	9	2.925	0.0948
FC-B - A-30	3.623	3.12	9	1.163	0.7711
FC-F-S - FC-F	-0.119	3.85	9	-0.031	1
FC-F-S - A-70	5.465	2.96	9	1.847	0.4058
FC-F-S - A-30	1.078	3.31	9	0.326	0.9971
FC-F - A-70	5.584	3.89	9	1.434	0.6238
FC-F - A-30	1.196	4.17	9	0.287	0.9982
A-70 - A-30	-4.387	3.36	9	-1.307	0.6942

Table A8: Pairwise comparison of anode slopes, reactors grouped by cathode group

Table A9: ANOVA for F cathode slopes, reactors grouped by cathode group

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
wc_curdens_mean	1	490.4	490.4	37.206	0.001715
cat_group	2	15261.9	7630.9	578.938	1.21E-06
wc_curdens_mean:cat_group	2	644.7	322.3	24.456	0.00262
Residuals	5	65.9	13.2		

Table A10: Pairwise comparison of F cathode slopes, reactors grouped by cathode group

Cathode Groups	estimate	SE	df	t.ratio	p.value
FC-B - FC-F-S	7.42	1.9	5	3.914	0.0254
FC-B - FC-F	17.5	2.61	5	6.703	0.0026
FC-F-S - FC-F	10.07	2.73	5	3.693	0.0317

Table A11: ANOVA for A cathode slopes, reactors grouped by cathode group

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
wc_curdens_mean	1	3764.8	3764.8	60.487	0.001474
cat_group	1	2787.2	2787.2	44.7806	0.002594
wc_curdens_mean:cat_group	1	131.7	131.7	2.1164	0.21942
Residuals	4	249	62.2		

Table A12: Pairwise comparison of A cathode slopes, reactors grouped by cathode group

Cathode Groups	estimate	SE	df	t.ratio	p.value
A-70 - A-30	7.51	5.16	4	1.455	0.2194